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Hybridism and the Germ=Cell.

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The writer prepared a paper on "The Spermatogenesis of Hybrid and of Normal Pigeons" in May, 1900, and placed it with *The Journal of Morphology*. On account of the temporary suspension of that periodical it is deemed desirable to publish an abridged account of the results of the investigation,* leaving details to appear in the original paper whenever *The Journal* shall resume publication. The chief interest of the investigation centers in the peculiarities met with in the transformation of the germ-cells of hybrid pigeons, hence the present paper will emphasize this phase of the subject.

The germinal cells of the male pigeon are laid down in a great number of delicate convoluted tubules, which wind back and forth throughout the interior of each testis and make up its main bulk. Near the periphery of each tubule are the spermatogonia, or parent cells (Fig. 1, sg.), which through growth and division give rise to the various generations of germ-cells lying inward toward the lumen of the tubule. The adult spermatozoa are formed through the final transformation of the spermatids, or cells which lie nearest the center of the tubule, the product of the last cell-division. As in many forms, the spermatozoa attach themselves to a supporting cell (Fig. 1, s.) for a period before their complete maturation and ejection from the testis.

The usual four phases or types of the germinal cells are recognizable, viz.: (1) spermatogonia (Fig. 1, sg.), a more or less regular layer of cells lying next to the wall of the tubule, each cell of which through division gives rise to two new cells.

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* The original paper is the thesis submitted by the writer as a candidate for the degree of Ph.D., at the University of Chicago. It was accepted by the faculty in March, 1900. A few copies of a limited edition of the thesis, published by the author, are available for investigators who are specially interested in the particular problems under discussion. Address the writer at the *University of Cincinnati*.

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One or both of these may increase in size and become (2) primary spermatocytes (Fig. 1, scy. 1), or remain in the layer and continue as spermatogonia. The primary spermatocytes, after some interesting changes, divide to form (3) the secondary spermatocytes (Fig. 1, scy. 2), which divide again shortly and give rise to (4) the spermatids (Fig. 1, st.), through the transformation of which the spermatozoa are developed. The number of chromosomes in each type as seen at the equator of the spindle before division is, in the spermatogonia sixteen loops, in primary spermatocytes eight rings, and in secondary spermatocytes four rings. The nurse cells, or Sertoli cells, to which the spermatozoa become attached at one period of their transformation (Fig. 1, s.), are irregularly disposed among the other cells.

THE SPERMATOGONIA.

The spermatogonia lie in a more or less regular layer along the wall of the tubule. In early stages they are far apart and possess small nuclei, which are oblong with the long axis parallel to the tubule wall. The cell boundaries are at first very indistinct or seemingly absent, and gaps frequently intervene between the individual spermatogonia, so that the latter seem to have been left behind from a preceding set, or to have recently settled in their present position. In later stages the cells are crowded together till they become columnar in shape, while the nuclei increase in size and become very distinct. A condensation of the cytoplasm, or mass of sphere substance (idiozome of Meves), makes its appearance, and gradually increases in size till it becomes a well defined area (Fig. 2, i). Near the center of this mass is generally a clear area, in which a minute centrosome (c) is discernible. A nucleolar-like mass is usually visible within the nucleus, but judging from its reactions to various stains, it is nothing more than a clump of the same material that composes the linin. This mass usually takes part, together with the linin network, in forming the achromatic sheath within which each individual chromosome is incased when ready for division. Before division the nucleus passes through an incomplete spirem stage. The spirem breaks up into individual chromosomes, which are visible as irregular threads and loops scattered throughout the nucleus. A filament two or three times as long as the other chromatic bodies is to

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be seen at times. Before the formation of the spindle for the ensuing cell division, this body is ejected into the sphere substance, where it seems to ultimately break up and become scattered throughout the cytoplasm. The significance of this phenomenon could not be determined. It seems improbable at present that the extruded body can be homologized with the "accessory chromosome" of McClung[1], a curious nuclear element, which he describes as occurring in *Xiphidium fasciatum*, one of the *Locustidae*. The "accessory chromosome," according to his account, does not disintegrate and disappear, but retains its individuality, and persists throughout the entire period of spermatogenesis, to take part finally in the formation of the spermatozoon.

After the formation of the chromosomes, the centrosome, which lies in a clear area of the sphere, divides into two, one of which moves along the outer periphery of the nucleus to the opposite pole. The first appearance of the spindle fibers is as radiations which spread around the nucleus from the centrosomes. When the mitotic figure is fully formed, the spindle is short and broad and the chromosomes lie in a confused band at the equator (Fig. 3). The individual chromosomes are loop-shaped, with the closed end of the loop toward the center of the spindle. While moving toward the poles after division, not infrequently the free ends of a chromosome fuse to form a small ring. After repeated attempts at counting, it was determined that sixteen chromosomes are present at the equator of the spindle before division.

PRIMARY SPERMATOCYTES.

The primary spermatocytes originate from the cells of the last spermatogonial division through a process of growth. The chromatin passes into the resting condition, and an increase in bulk of both the nucleus and the cytoplasm begins. The sphere first appears as an indistinct granular crescentic area closely applied to the nucleus, with the horns of the crescent so extended as to inclose more than half of the nuclear surface. As the young spermatocyte grows, the sphere also increases in

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[1] McClung, E. C. A Peculiar Nuclear Element in the Male Reproductive Cells of Insects. Zoöl. Bul. II. 4, 1899.

size, becoming more and more rounded. From an early stage a minute centrosome is visible in the midst of the sphere substance. It is surrounded by a clear area, which becomes more pronounced as the sphere grows older. Thus the developing cell gradually acquires characteristics of size, shape and general appearance, that differ markedly from those of the previous generation.

Synapsis.— Synapsis occurs in the primary spermatocytes, during which there is a marked drifting of the chromatin to the side of the nucleus in contact with the sphere (Fig. 4). Some substance from the nucleus apparently passes out into the sphere; it may possibly be concerned in the formation of the extremely coarse-fibered spindle, for almost immediately the centrosome divides and the spindle appears. In the ensuing division of the spermatocyte only eight chromosomes are present, but they are in the form of heavy rings, and are evidently bivalent (Fig. 7).

During division the eight-ring chromosomes, which are incased in capsules of linin, break transversely, and as they move apart remain connected by threads of the linin casing. These threads constitute the interzonal fibers (Fig. 8, if). An intermediate body is present at the equator of the interzonal fibers and marks out the path of the new cell walls (Fig. 8, ib). The ring type of chromosome seen at this division is formed through the breaking up or rearrangement of the prominent spirem (Fig. 5), which forms immediately after synapsis. The spirem-like appearance inside the nucleus disappears gradually, until by the time the centrosomes reach their positions at opposite poles of the nucleus, the components of the spirem are seen as eight elongated, irregular rings (Fig. 6), which consist of a linin groundwork, in which are imbedded numerous granules and lumps of chromatin. The rings gradually condense into a shorter, heavier type, and the chromatin fuses in such a way that distinct granules are no longer visible. In a few instances rings were found to consist of four more or less spherical, densely staining areas, connected by lighter bands of linin. It is possible that this is comparable to the tetrad formation so frequently observed in maturation phenomena (Fig. 6, tr).

The product of the division just discussed consists of two cells, each of which is considerably smaller than the primary spermatocyte, and which never attains to its volume. These cells are the secondary spermatocytes. They go into a resting stage, which is of very short duration. When the secondary spermatocyte is ready for division, curiously enough only four chromosomes appear (Fig. 9). They are of the same shape and size as those in the division of the primary spermatocyte. In dividing, the chromosomes each break in such a way that a stringing out of the sheaths of the chromosomes gives rise, as in the primary spermatocytes, to a system of interzonal fibers, which, as division proceeds, constrict at the equator to form a large intermediate body. For some time after division the divided chromosomes are seen as four hollow vesicles within the daughter nuclei. They fuse later, ordinarily, into one large, hollow sphere of chromatin near the center of the nucleus (Fig. 10). Numerous fine fragments of chromatin migrate to the nuclear membrane, which has appeared in the meantime, and form a thin shell of chromatin along its inner surface. The centrosome (Fig. 10, c) persists, and together with the tip of the spindle moves out into the cytoplasm. The tip of the spindle seemingly becomes re-converted into sphere substance.

THE SPERMATID AND ITS TRANSFORMATION.

The new cell formed from the division of the secondary spermatocyte is the spermatid, and is the cell which will ultimately be transformed into the spermatozoon. An adult spermatozoon as it exists in the vas deferens of the pigeon is shown in Fig. 14. The head is long and narrow, and is intensely stained by nuclear dyes. Favorable preparations show the chromatin arranged in a series of vesicles within the head. Each vesicle of this chain-like series incloses a clear area, which in some preparations appears highly refractive. A remarkable fact is that the number of vesicles is apparently the same as the reduced number of univalent chromosomes should be, namely, eight. In some instances, where only six or seven vesicles were present, it was observed that one or two were unusually large, and hence probably equivalent to two. It will be recalled that

in the secondary spermatocyte there were only four chromosomes, but that they were of the bivalent type, or really comparable to eight ordinary chromosomes. There is no positive evidence that the vesicles in the head of the spermatozoon correspond to individual chromosomes, but the striking coincidence in number is at least very suggestive, and it would not be surprising if the fact develops later that after the entrance of the spermatozoon into the egg the vesicles resolved themselves into eight distinct chromosomes.

At the anterior end of the head is a slender, fine-pointed head-spine. The head posteriorly connects directly with the long cytoplasmic tail. No middle piece is visible. The tail and the head-spine are very difficult to observe accurately, and but little of the details of their structure could be worked out. The only way to gain a satisfactory knowledge of the spermatozoon at all is through a study of its development.

In the transformation of the spermatid to form the spermatozoon the first change to be observed is in the centrosome. It divides, and one of the resulting centrosomes enlarges and becomes ring-shaped (Fig. 11, c). The axial filament of the tail first appears as a thread connecting the two centrosomes, and later continues backward through the ring-like centrosome and out of the cell (Fig. 11, ax). The smaller centrosome, together with material of cytoplasmic origin, finally comes to lie within the nuclear membrane. It may be regarded perhaps as a middle piece, which becomes obscured by a covering of chromatin, and consequently appears to be absent in the adult spermatozoon.

The long head of the spermatozoon is the transformed nucleus. In the process of elongation only what may be termed the anterior and the posterior ends of the nucleus extend at first, but in a short time the entire nucleus begins to narrow. At the same time the mass of chromatin at the center sprouts out both anteriorly and posteriorly to form a central, thread-like core (Fig. 12). As the process of elongation continues, a narrowing of the sides of the nucleus takes place to some extent, but when one takes into account the enormous elongation that occurs, together with the relatively slight diminution of the transverse diameter, it becomes evident that there must be considerable increase in the volume of the nucleus. The heavy central chromatic filament after a time becomes arranged in a wavy

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or spiral manner. As the transformation progresses the spiral design, although often very irregular, becomes more perceptible. A splitting of this spiral core finally occurs, and thereafter the chromatin exists as two threads laid down in an irregular double spiral (Fig. 13).

The elongation of the nucleus ceases at about the time the bisection of the central filament has been accomplished, and the nucleus displays itself as an enormously long, sinuous head, which may measure twice the length of the head of the adult spermatozoon. A dense protoplasmic mass encases it and extends backward along the axial filament. A shrinkage of the nucleus follows, in the course of which the double spiral of chromatin shortens and widens until the exact relationship of the chromatin of the two filaments can no longer be determined. The final appearance is that of a chain-like series of vesicles, as described for the mature spermatozoon (Fig. 14), the clear area in the center of each vesicle corresponding to the openings between the respective points of intersection of the two spirals of chromatin.

The head-spine originates from a bubble-like mass of material (Fig. 11, v) which arises in the sphere. This bubble or vacuole moves slowly around the periphery of the nucleus until it lies at the pole opposite the point at which the centrosome, which marks the anterior end of the axial filament, will enter the nucleus.

THE GERM-CELLS OF HYBRID PIGEONS.

In the pigeon some crosses are fertile, others are not. The sterile hybrids show a greater or less degeneration of the germinal cells. The general rule seems to be that the more divergent the parent forms, the more marked is the degeneration of the germinal cells. From parents which differ widely in structure or habits there seems to be much greater difficulty in securing female than male offspring. I have been able to obtain but one female offspring of very distinct species for microscopical examination, while, on the other hand, I have had six males. For all of this material I am indebted to Professor Whitman. From the testis of the offspring of the common ring dove, *Turtur risorius,* and the white ring dove, *Columba alba,* a large number of sections were made for microscopical study. These two

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forms are perfectly fertile when crossed, and the fertility of their offspring seems in no wise diminished. The germ-cells show some of the same phenomena as those of the sterile birds, though in a less marked degree.

The common brown ring dove when crossed with the white ring dove produces brown offspring. One member of the resulting pair is usually a few shades lighter in color than the other. In the next or third generation there is generally a return to the original colors of the grandparents; one of the young is white, the other brown. There is a marked tendency for the white ones to be female and the brown ones male, this being true at least of the nine pairs killed by the writer. Occasionally in the third generation both of the young are white or both brown.

Of the sterile hybrids, whether male or female, the sexual products were abnormal. The abnormalities of male hybrids may be classified conveniently under three heads: (1) Abnormalities in mitosis; (2) abnormalities in the structure of the spermatozoon; (3) degeneration of the germinal cells. Not all hybrids show these various irregularities in the same degree. All three kinds of the phenomena just mentioned are observable in the sterile forms, but the fertile birds differ, for the most part, from normal pigeons only in the slightly irregular character of the mitosis.

The *abnormalities in mitosis* are in the nature of multipolar spindles and asymmetrical division and distribution of the chromosomes (Figs. 15-19). They are more pronounced in sterile birds, but may be met with in fertile hybrids also. It is a curious fact that the multipolar spindles are confined largely to the primary spermatocytes, and one is inclined immediately to associate the fact with the pseudo-reduction or formation of bivalent chromosomes, which occurs normally at this stage of spermatogenesis. Figs. 15-18 show some of the different forms of multipolar spindles. The tripolar types are by far the most common. Fig. 15 represents perhaps the most prevalent structure. It was not unusual to observe two spindles in one cell, as shown in Fig. 16. When two such spindles exist independently in one cell, they may each have a small number of the large bivalent ring-form chromosomes, or a greater number of small chromosomes, which are apparently univalent. More rarely both large and small chromosomes occupy one or both

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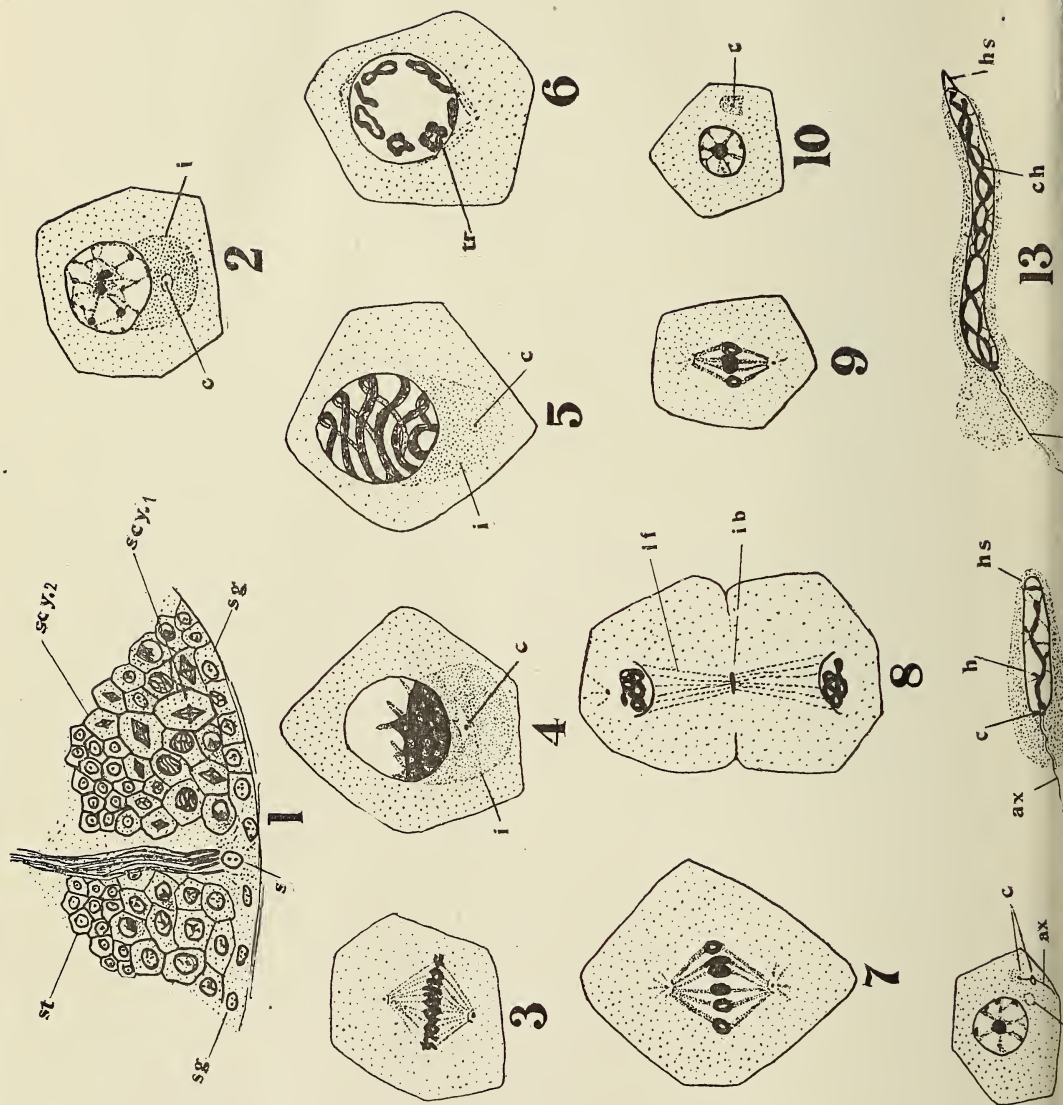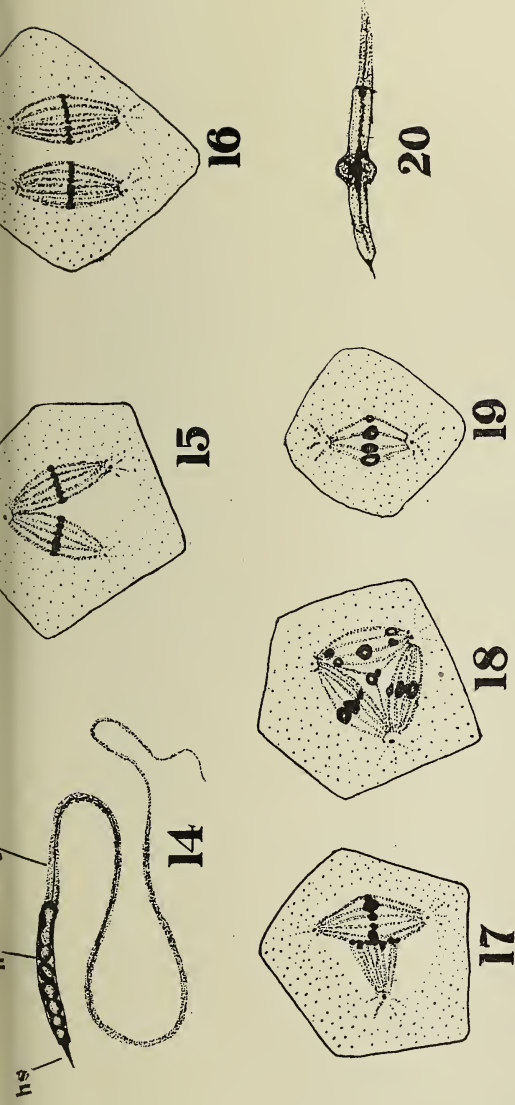
EXPLANATION OF FIGURES.

Fig. 1. Section of a tubule showing various stages in the maturation of the spermatozoön. *s*, Sertoli cell; *sg*, spermatogonia; *scy.* 1, primary spermatocyte; *scy.* 2, secondary spermatocyte; *st*, spermatid.

Fig. 2. A spermatogonium. *i*, sphere (idiozone); *c*, centrosome.

Fig. 3. A spermatogonium ready for division.

Fig. 4. A primary spermatocyte in synapsis. *i*, sphere; *c*, centrosome.

Fig. 5. Primary spermatocyte in the spirem stage.

Fig. 6. The formation of ring chromosomes in the primary spermatocyte.

Fig. 7. Primary spermatocyte ready for division.

Fig. 8. Division of primary spermatocyte nearing completion. *if*, interzonal fibers; *ib*, intermediate body

Fig. 9. Secondary spermatocyte ready for division.

Fig. 10. Spermatid. *c*, centrosome lying in the persisting tip of the spindle.

Fig. 11. Spermatid. *ax*, axial filament; *c*, centrosomes; *v*, vacuole (head-spine).

Fig. 12. Elongation of the nucleus of spermatid to form the head of the spermatozoön. *ax*, axial filament; *c*, centrosome; *h*, head; *hs*, head-spine.

Fig. 13. Continuation of process shown in Fig. 12. *ch*, central core of chromatin which has split to form double spiral.

Fig. 14. Mature spermatozoön. *h*, head; *hs*, head-spine; *t*, tail.

Figs. 15-18. Irregularities in division of the primary spermatocytes found in hybrids.

Fig. 19. Secondary spermatocyte of hybrid, showing three normal and one dwarf chromosomes.

Fig. 20. Abnormal spermatozoön found in sterile hybrids. Most of the tail is omitted in the drawing.

of the spindles. The facts indicate that a pseudo-reduction has not occurred, or that it is incomplete or abnormal. What has just been said regarding the chromatin arrangement, where two separate spindles occur, is equally applicable to the multipolar forms, only there is generally more variation in the size of the chromosomes. In a tripolar type like Fig. 15 the chromosomes are almost invariably numerous and of small size.

In a few instances two nuclei were present in the primary spermatocyte, and it seems probable that such cells give rise to the multipolar spindles, bearing an excessive amount of chromatin, which are sometimes seen. Fig. 18 shows a tripolar spindle in a primary spermatocyte, where there is much variation in the size of the chromosomes.

An asymmetrical distribution of chromatin results, of course, in many cases where the division is by means of multipolar spindles, but in addition to this there is very frequently an unequal division of the chromosomes themselves. This occurs as often where the spindle is single as in any other case. In dividing, perhaps only one-quarter of a chromosome will go to one pole and the other three-quarters to the opposite pole, or the division may be such that a portion of the chromatin is cut out entirely and left behind in the cytoplasm. Fig. 19 represents a secondary spermatocyte, of which one chromosome is very minute, as if part of its material had been lost in the preceding division.

In two or three instances one of the large chromosomes of the primary spermatocyte was observed to be made up of four small rings. Whether this indicates an exaggerated demarcation into tetrads it is impossible to affirm, though the fact is a significant one.

Abnormalities in the structure of the spermatozoa are present in sterile hybrids. There is a curious varicosity about the middle of the spermatozoon head in such forms that attracts the attention immediately, when the objects are examined under the microscope (Fig. 20). This enlargement seems to be almost universal amongst the spermatozoa, and is sufficient of itself to produce sterility, for such a malformation would prevent its possessor from entering the egg. In a very few instances what appears to be a normal spermatozoon may be observed among the misshapen ones, and it is possible that if these reached a

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suitable egg, fertility might result. Their chance of meeting with the egg, however, is very slight.

A study of the development of these deformed spermatozoa reveals the fact that the bead-like enlargement results from the incomplete development of the nucleus in the formation of the head. The two ends of the nucleus sprout out, as it were, and grow for a short distance, but the remainder of the nuclear-wall retains its original form and position. The arrangement of the chromatin is very irregular. A deeply staining mass is visible in the bulb-like swelling, from which thick filaments spring out forward and to the rear.

Degeneration of the germinal cells was in progress in the testes of all sterile forms, but was most pronounced in hybrids from birds which were of very divergent species, or hybrids which were themselves descendants of fertile hybrids. There were some cases of such extreme degeneration that only the layer of cells lying along the wall remained in the tubule. Where such a degree of degeneration exists there is of course no approach to the formation of spermatozoa. There is often a strong invasion of wandering cells into the tubules, especially where the degenerative activities have become extensive. The interspaces between such tubules are also usually packed with cells which resemble white blood corpuscles. In some preparations it looks really as if the germinal cells themselves lose their walls and characteristic appearance and become leucocytes, though no definite conclusions could be reached regarding this point. Some tubules are occupied almost entirely by cells which have the exact appearance of the large stroma cells present outside the tubules.

The primary spermatocytes seem to be the cells most susceptible to decay. Frequently the nuclear contents have a watery and disintegrated appearance and the sphere substance is marked by the presence of a large vacuole in its center. In testes where degenerative processes are pronounced a number of cells may run together to form a giant cell, in the center of which is an enormous vacuole surrounded by the nuclei of the original cells. The mass thus formed is very similar in appearance to the giant cells found in many pathological tissues.

A detailed description of the individual hybrids will not be entered into at present, but will be left to the original paper. The crosses were: (1) Male black tumbler, female brown ring

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dove; (2) male black tumbler, female brown ring dove; (3) male hybrid from white and brown ring dove, female homer; (4) male wild passenger pigeon, female brown ring dove; (5) male wild passenger pigeon, female brown ring dove; (6) male turtle dove, female Japanese turtle dove. The hybrid from the last named was a female. The ovary and oviduct were rudimentary. Occasional small ova with an incomplete follicle were present. The largest egg had attained to a diameter of only seventy-five micra; (7) numerous crosses of white and brown ring doves.

CONCLUSIONS FROM THE STUDY OF HYBRIDS.

It appears that in crosses of very divergent species the degenerative processes in the germ-cells are at a maximum. In closely related forms like the brown and the white ring doves fertility is not diminished, and the testes seem to be normal, except for occasional irregularities in mitoses.

The formation of multipolar spindles in division and the unequal distribution of chromosomes seen in many instances are among the most interesting phenomena presented. Such irregularities, however, occur to a slight extent in normal birds. A very careful study reveals the fact that they are more prevalent in the ordinary dove-cot pigeons than in pure breeds of doves, which have bred true for many generations.

That the irregularities of division in hybrids are due to degenerative processes going on in the tubules, which give rise to deleterious chemical substances, is the first thought that presents itself. This seems a very acceptable idea from what we know of the effects of drugs upon cell division, but it does not account for the fact that the primary spermatocytes are the cells attacked in the great majority of cases.

A hybrid offspring is really a compound of two very different individual plasmas, hence conflicting tendencies must necessarily have been induced within its body. The abnormality in division may be but an attempt of each plasma to assert its individual activity. But why does this effort become apparent only in the primary spermatocytes? The answer appears to be simply that there is no necessity for fusion of the components of the chromatin of cells except in the germ-cells at one stage of their maturation. Investigators have found that in matura-

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tion of germ-cells a reduction of the ordinary number of chromosomes to one-half occurs. Before this actual reduction there is ordinarily a so-called pseudo-reduction, in which the chromosomes fuse in pairs, so that when the cell is ready for division, although only half of the regular number of chromosomes appear, each is really double (bivalent), and equivalent to two of the simple (univalent) type. In spermatogenesis this change generally comes about in the primary spermatocyte. Now, in hybrids it may be supposed that in the ordinary cells of the body the chromosomes from the paternal and the maternal species lie side by side and carry on the customary functions of the cells, but when it comes to an actual fusion of chromosomes to form the bivalent type necessary for reduction, the incompatibility of the two different plasmas renders the union incomplete or prevents it entirely. That the ordinary somatic cells of hybrids, either plants or animals, are under the influence of two distinct tendencies is well shown in the decided mosaic-like structures which frequently occur, the classic figure being to liken hybrids to warp and woof. To cite but one example, Macfarlane found that a hybrid of the gooseberry and black currant, instead of being a strictly intermediate type, really possessed, side by side, organs characteristic of each parent; the leaves bore both the shield-shaped, oil-secreting hairs of the currant and the simple hairs of the gooseberry, though each hair was but half the size of the parent type. We may infer, then, that in certain hybrid pigeons the univalent chromosomes from each of the parents may lie side by side in the ordinary cells of the body and divide normally, but when it comes to the period of fusion in the germ-cell, they will not unite to form the bivalent type, or else they unite incompletely. The result is that in the primary spermatocyte, instead of one spindle bearing eight bivalent chromosomes, a multipolar spindle, or not infrequently two separate spindles, bearing two groups of univalent chromosomes, may appear. In cases where both large and small chromosomes are seen, it is necessary to suppose that a loose union has occurred in some chromosomes. The unequal divisions of the bivalent chromosomes of many hybrids indicate that such chromosomes have in some way been rendered very unstable.

If we accept the view that chromatin is a substance capable of varying in qualities in the different regions of the chromo-

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some, then in fertile hybrids, where irregular mitoses occur, the different germ-cells will certainly not be qualitatively similar after division, and one would expect the offspring produced from such cells to be variable. That the chromatin of each parent species often retains its individuality is indicated by the fact observed in many primary spermatocytes where two separate groups of the small or single type of chromosome exists. The division of such a cell into three or four, as the case may be, results in the formation of new cells, some of which will manifestly contain chromatin from only one of the original parent species, and some only from the other. Some of the spermatozoa, then, will bear chromatin from only one of these species. In the offspring from such a cell one would expect a much closer return to whichever one of the parent forms it represented than in the offspring of a "mixed" spermatozoon.

In discussing irregular divisions, however, it must not be forgotten that many apparently normal divisions of the primary spermatocytes also occur in all hybrids, and constitute by far the predominant kind of division in hybrids from closely related forms. Unequal distributions of chromatin cannot therefore play the most important part in variation or reversion. There seems to be no other interpretation, indeed, than that in the many *normal* mitoses of the bivalent chromosomes which occur, the chromatin of the father and of the mother is set apart so that the ultimate germ-cells are what might be termed "pure" cells; that is, a given egg or sperm-cell contains exclusively or at least predominantly qualities from one parent. The offspring from fertile hybrids of the same parentage might then be similar to the mixed type of the original hybrid, or revert to one of the grandparent types, dependent upon the chances of the various cells for union at fertilization. If a spermatozoon and an egg containing characteristics of the same species unite, then the reversion will be to that species; if a sperm-cell containing the characteristics of one species happens to unite with an ovum containing characteristics of the other species, then the offspring will be of the mixed type again. By the law of probability the latter will be the more prevalent occurrence, because there are four combinations possible, and two of the four would result in the production of mixed offspring, while only one combination could result in a return to one of the ancestral species.

From the fact that in cases of apparently complete return to one parent type, characteristics of the other parent may nevertheless crop out from time to time in succeeding generations, it is evident that all of the germ-cells are not absolutely "pure." The occasional inequalities in the division of individual chromosomes, as already mentioned, may account for this fact. It is probable that the irregular distributions of chromatin, where such occur, have more to do with such succeeding offspring as show variation, and less with those which return to the specific types. In the latter case the chromatin of each species has remained entirely distinct (in *marked* hybrids), or has normally separated again at the sundering of the bivalent chromosomes (in *mild* crosses) into the two original plasmas. It is very obvious, of course, that most of the variation seen in the off- spring of fertile hybrids is due to the union again of two "pure" germ-cells, each of which represents a different one of the original parent species.

That *irregular* divisions can not account entirely for reversions to grandparent types is very evident in the crosses of brown and white doves, where the irregularities are by far too few to equal the percentage of reversions. There is but one ultimate conclusion, then, namely, that the irregularity in division of the primary spermatocytes, which appears in hybrids between very different species, is *but an index* to what occurs in ordinary crosses. In the latter, instead of separate spindles and non-fusion of chromosomes, a true union occurs, but the bivalent chromosomes ultimately divide in such a way that the respective plasmas occupy different cells. *There is a separation of the paternal and the maternal chromosomes which had fused during synapsis.*

With regard to the question of the persistence of chromosomes, the evidence is becoming stronger every day that these elements do retain their individuality. Rückert,[2] for instance, in his study upon the fertilization of cyclops, was able to follow the maternal and paternal chromosomes very distinctly in cleavage. Again, to cite only one or more of the rapidly multiplying examples, Herla[3] and Zoja[4] have shown that in the hybrid fer-

2 Rückert, J. Zur Eireifung bei Copepoden: Qu. Hefte, 1894.

3 Herla, V. Étude des variations de la mitose chez l'ascaride megalocephala: Arch. Biol., XIII., 1893.

4 Zoja, R. Sullo independenza della cromatina paterna e materna nel nucleo delle cellule embrionali: Anat. An. XI., 1895.

tilization of *Ascaris*, if the eggs of variety *bivalens* is fertilized with the spermatozoon of variety *univalens*, the three chromosomes thus brought together retain their individuality and reappear at each cleavage, at least to the twelve-cell stage. Zoja affirms that the paternal chromosome is of smaller size and is thus distinguishable from the two maternal chromosomes.

The above interpretations are offered with the hope that they may perhaps lead to some clew concerning the real nature of the material basis of heredity. If the conception proves to be a true one, then it doubtless affords a key, among other problems, to the long-standing one as to why many plants will come true from slips or grafts, but not from seed. The reason may be sought in the pseudo-reduction period of the germ-cell. Plants such as the apple, for example, which do not come true from seed, are practically multi-hybrid. In the germ-cells there will be numerous incompatibilities due to the fact that the plant has been miscellaneously fertilized for a number of generations. In propagation by means of slips, the chromosomes lie side by side and divide in the ordinary way to construct and maintain the new body, so that it is practically a continuation of the old one; but when the time comes for maturation of the germ-cells, the lack of harmony between the various plasmas represented asserts itself, with the result that bivalent chromosomes are formed, which divide in such a manner as to segregate different sets of ancestral qualities. The resulting combinations in fertilization will give rise to seed many of which may possess dissimilar sets of qualities.

As to the other abnormalities met with in the spermatogenesis of hybrids, about all that can be said is that the whole phenomena show lack of vigor in the development of the germ-cells, whatever this may mean. The deformed spermatozoa indicate want of sufficient vitality to push the development through to completion. The germ-cells start out apparently to perform their functions normally, but later succumb to the conflicting forces at work within their boundaries.

As to why the reproductive organs should be more susceptible to abnormal changes than other regions of the body, we have no clew. Darwin has pointed out repeatedly the curious parallel between crossing and the change produced by physical conditions. Animals and plants removed from their natural environment are extremely liable to have their repro-

ductive systems affected. Still he recognizes that sterility is incidental and not a necessary concomitant of hybridism. Hybridization in some forms, indeed, increases fertility.

SUMMARY.

1. The usual four types of germinal cells are recognizable, viz: (1) spermatogonia, (2) primary spermatocytes, (3) secondary spermatocytes, and (4) spermatids. Sertoli or nurse cells are likewise present.

2. The number of chromosomes in the spermatogonia is sixteen, in primary spermatocytes eight, and in secondary spermatocytes four.

3. The spermatogonia vary considerably in appearance at different phases of their activity. A sphere (idizome), within which the centrosome lies, is visible before division.

4. A filament two or three times as long as the other chromatic bodies in the nucleus is cast out into the cytoplasm before the formation of the spindle for division of the spermatogonium.

5. Synapsis occurs in the primary spermatocytes, during which there is a marked drifting of the chromatin to the side of the nucleus in contact with the sphere.

6. At the division of the primary spermatocyte, only eight chromosomes are present, but they are in the form of heavy rings, and are evidently bivalent.

7. In division the chromosomes break transversely, and as they move apart, remain connected by threads of the linin casing which encapsuled the chromosomes. These threads form the interzonal fibers.

8. Intermediate bodies are present at the equator of the interzonal fibers, and mark out the path of the ensuing division of the cytoplasm.

9. At the division of the secondary spermatocytes, the four chromosomes which appear are of the same size and shape as those of the preceding division.

10. In the transformation of the spermatid the first perceptible change is in the centrosome. It divides, and one of the resulting centrosomes enlarges and becomes ring-shaped. The axial filament of the tail first appears as a thread connecting the two centrosomes, but later continues backward through the ring-like centrosome and out of the cell.

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11. The smaller centrosome, together with material of cytoplasmic origin, finally comes to lie inside of the nuclear membrane. Although a middle piece appears to be absent in the adult spermatozoon, it seems probable that this centrosome within the nucleus may function as a middle piece which has become obscured by a covering of chromatin.

12. The nucleus elongates to form the long head. It contains a central core of chromatin in the form of a spiral filament, which splits later to form a double spiral.

13. The head, during the later stages of development, undergoes a very great contraction, but the spiral arrangement of the chromatin still persists in a modified form.

14. The chromatin appears finally to be arranged within the head in a series of vesicles. A remarkable fact is that the number of vesicles is the same as the reduced number of univalent chromosomes should be, namely, eight.

15. The head-spine originates from a bubble-like mass of material which arises in the sphere of the spermatid.

16. The general plan of spermatogenesis in hybrid pigeons is not essentially different from that of normal pigeons.

17. All hybrid pigeons exhibit multipolar spindles and asymmetrical distributions of the chromatin in cell division. These irregularities are much more infrequent in fertile hybrids.

18. Infertile hybrids show in addition a deformed spermatozoon, and often a marked degeneration of the germinal cells.

19. The irregularities of division are confined for the most part to the primary spermatocytes. Likewise it is in these cells that the formation of bivalent chromosomes occurs normally. In hybrids, it would seem that the conflicting tendencies of the two parental plasmas frequently render the union of the single chromosomes to form the double (bivalent) types impossible or abnormal. There seems to be an attempt on the part of each plasma to assert its individuality. This visible incompatibility of the chromosomes from widely different species serves *as an index* to a kindred lack of harmony between the plasmas of more nearly related forms, so that even though pseudo-reduction does occur and normal division of the bivalent chromosomes follows, the identity of the individual species is still retained through the segregation of the maternal and paternal chromosomes into separate cells, which may be considered "pure" germ-cells (containing qualities of only one species).

20. Union of two cells containing characteristics of the same species would occasion a reversion to that species. Union of two cells representing each of the two original species would yield an offspring of the mixed type. The latter would predominate because of the greater probability of such union. Besides, through the mixing just indicated, variability may be due also in some cases to the not infrequent inequalities in the division of individual chromosomes, through which varying proportions of the chromatin of each species may appear in certain of the mature germ-cells.

21. *Irregular* divisions can not of themselves account entirely for reversion and variations, because double spindles and irregularities in the formation of bivalent chromosomes are by far too few to equal the percentage of reversions seen in such mild crosses as the brown and the white ring dove. One is forced to the conclusion expressed above, that the double-spindled and multipolar types of cells which occur in hybrids between very divergent forms are but exaggerated images of a tendency which exists in the primary spermatocytes of normal appearance, which are to be found in all hybrids.

22. The above conception may likewise afford a clew to the problem of why certain plants will come true from slips or grafts, but not from seed. The explanation may be sought in the pseudo-reduction period of the germ-cell.
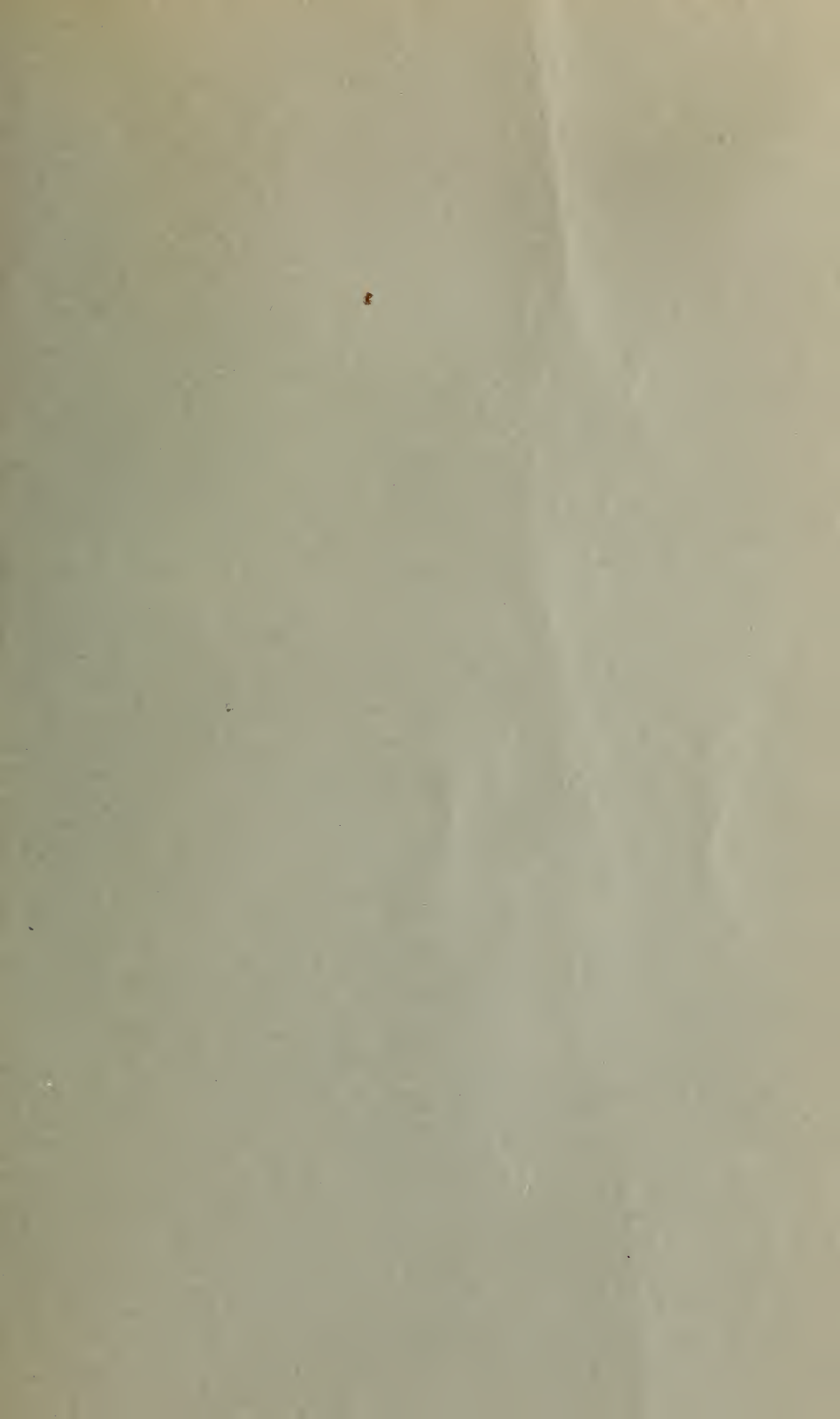
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Note.—Inasmuch as the present paper is a resumé of the writer's thesis of 1900, it is not deemed advisable to enter into a discussion of any of the more recent papers which have a bearing on the results obtained. Juel's paper on hybrids of *Syringa*, which did not appear until later, is perhaps the most significant. His hybrids exhibited abnormal mitoses similar to those that are found abundantly in *pronounced* pigeon-hybrids. From his facts I would suggest that the same interpretation as set forth in my conclusions holds true, although it can not be extended in the same detail to fertile forms, because his hybrids were sterile. Two very brief abstracts of one or two of the more unusual points presented in the writer's thesis have appeared in *Science*, but thus detached from the body of the work, it would seem from the tenor of letters which have been received that in some cases a misunderstanding of the writer's exact position has arisen. It is hoped that the present paper will set forth the main facts in their true perspective.